A PRELIMINARY REPORT OF MESCALINE CONCENTRATIONS IN SMALL REGROWTH CROWNS VS. MATURE CROWNS OF *LOPHOPHORA WILLIAMSII* (CACTACEAE): CULTURAL, ECONOMIC, AND CONSERVATION IMPLICATIONS

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ABSTRACT

A phytochemical analytical study was conducted to address the question of whether the mescaline concentration in *Lophophora williamsii* (peyote) is dependent on the maturity and/or size of the plant. Samples of crown tissue (4 g each) biopsied from mature peyote cacti and whole small regrowth crowns (2–4 g each) were collected from the same population in the Tamaulipan Thornscrub ecoregion of South Texas. For each of the two groups (mature and small regrowth), the individual tissue samples were pooled, desiccated, and ground to powder. The alkaloids were extracted with methanol at 25°C, followed by evaporation of the methanol to dryness, then acid-base cleanup with water and dichloromethane. The mescaline concentration in each of the extracts was then determined by HPLC. Quantitative analyses provided evidence that the small crowns that develop in response to harvesting contain a lower mescaline concentration—about half as much—compared to that of crowns of mature unharvested plants in the same population. The deficiency in the mescaline concentration of these regrowth buttons (new crowns) exacerbates the problem posed by the small size of the buttons; that is, it further increases the number of buttons that must be consumed to obtain an efficacious dose for ceremonial use by members of the Native American Church (NAC). That means that either the NAC members must consume less than the traditional amount of peyote, or there will be increased demand for peyote. Any increase in demand, reflected in the price, will engender more intensive harvesting, which will inevitably have adverse effects on both the supply of sacrament for the NAC and the conservation status of *L. williamsii* wherever the harvesters have access to peyote populations.

KEY WORDS: peyote conservation status, Native American Church, peyote overharvesting, dosage of mescaline, dosage of peyote, ethnopharmacology

RESUMEN

Se hizo un estudio fitoquímico analítico para determinar si la concentración de mescalina de *Lophophora williamsii* (peyote) depende de la madurez y/o el tamaño de la planta. Se recogieron muestras de tejido de la corona (4 g cada una) tomadas por biopsia en individuos maduros de peyote y muestras de coronas pequeñas enteras recrecidas (2–4 g cada una) de la misma población en la ecorregión del matorral espinoso tamaulipeco en el sur de Texas. Para cada uno de los dos grupos (maduros y pequeños recrecidos), las muestras individuales de tejido fueron combinados, secados, y molidos hasta polvo. Los alcaloides fueron extraídos con metanol a 25°C, seguido de evaporación del metanol hasta sequedad, y después una limpieza ácida- alcalina con agua y diclorometano. La concentración de mescalina en cada uno de los extractos fue determinada entonces por HPLC. Los análisis cuantitativos mostraron que las coronas pequeñas que se desarrollan como consecuencia del la cosecha contienen una concentración de mescalina más baja—aproximadamente la mitad—en comparación a la de coronas de plantas maduras no cosechadas en la misma población. La deficiencia en la concentración mescalínica de estas coronas recrecidas exacerba el problema causado por el tamaño pequeño de las mismas; es decir, aumenta aún más el número de "botones" (coronas) que tienen de consumirse para obtener una dosis eficaz para el uso ceremonial por miembros de la Native American Church (NAC). Eso significa que o los miembros de la NAC tienen que consumir menos cantidad tradicional de peyote, o habrá un aumento en la demanda de peyote. Cualquier aumento en la demanda, reflejado en el precio, engendrará recolección más intensiva, la cual inevitablemente tendrá efectos adversos, tanto en la provisión del sacramento para la NAC como en el estado de conservación de *L. williamsii* dondequiera que los peyoteros tengan acceso a las poblaciones de peyote.

INTRODUCTION

Lophophora williamsii (Lem. ex Salm-Dyck) J.M. Coult. (Cactaceae)—commonly known as peyote—is a small, spineless, globular cactus of northeastern Mexico and adjacent Texas (Fig. 1). It is of cultural and economic importance for its use as the religious sacrament of the Native American Church (NAC). The harvested crowns of peyote plants, either fresh or dried, are ingested orally as the sacrament in NAC ceremonies. Although *L. williamsii* contains over 50 alkaloidal substances (Anderson 1996), such spiritual use of the plant is evidently based largely on the psychoactive properties of an adequate dosage of its principal alkaloid, mescaline (Huxley 1955). The supply of peyote to registered NAC members is available through regulated channels involving the Texas Department of Public Safety and a very small number of licensed peyote distributors—currently there are three—who purchase their supply from agents (peyoteros) who harvest peyote from wild populations in South Texas. The peyoteros are paid by the piece, according to the number of buttons harvested and delivered to the place of business of a peyote distributor.

Over the past few decades peyote has become scarce in many parts of its historical geographic range. The largest part of the reduction in peyote population size is clearly habitat destruction associated with urban sprawl and adverse agricultural practices, notably root-plowing, which uproots and kills peyote along with the native brush, effectively exterminating the peyote along with the associated plants of its natural habitat, so that the damage to peyote in a root-plowed tract is absolute and permanent. Another major cause of the decline of peyote is overharvesting of the plant for ceremonial use by the NAC. Overharvesting of peyote has several different adverse effects on the wild populations:

(1) It reduces the harvestable population size, and selectively removes the largest crowns first, as these are most valued in the peyote market.

(2) That reduces the quantitative reproductive output of the population, as a direct consequence of the removal of the largest crowns that produce most of the seed in an unharvested population. In terms of population genetics, while such selective harvesting of the largest plants may not have a marked short-term effect on population size (assuming good harvesting practices, benign weather, and consequently a low mortality rate in harvested individuals), it has the immediate effect of reducing the effective population size.

(3) Concomitantly, there is a qualitative genetic loss in the selective loss of seed production from the oldest individuals, which are ipso facto best adapted to local conditions. That loss may be temporary, if the harvested plants survive to produce regrowth buttons that are allowed to mature after a few years, or it may be permanent, if mortality occurs in the old plants due to repeated harvesting of regrowth buttons (Terry et al. 2012).

(4) The phenomenon of post-harvest regrowth of new crowns arising from areoles of the subterranean stem (Terry & Mauseth 2006) temporarily increases the number of crowns in the population, but severely decreases both the average size and the total combined weights of crowns in the population (Terry et al. 2011).

(5) The decreased size of peyote buttons available to the NAC means that an individual in an NAC peyote ceremony must consume more buttons to equal the weight of the smaller number of buttons that would be consumed if mature crowns were available. This leads to a vicious circle of more frequent harvesting to supply the demand for greater numbers of buttons, which leads to the early harvesting of yet smaller buttons—but now fewer, as the overharvested plants exhibit signs of decreased energy reserves for the production of more new crowns following repeated harvesting at two-year intervals (Terry et al. 2012).

(6) Looking at the quality of peyote for ceremonial use in purely pharmacological terms, there would appear to be yet another disadvantage to regrowth buttons for ceremonial use, apart from their small size, and that is the possibility that the dry-weight concentration of mescaline in the small regrowth buttons is substantially lower than in mature peyote crowns, which now constitute a minor percentage of the total offering in the regulated peyote market.

Accordingly, the purpose of the current study was to address the hypothesis that small regrowth peyote

Fig. 1. A mature eight-ribbed peyote cactus (diameter ca. 5 cm) in habitat.

crowns have lower concentrations of mescaline (on a dry-weight basis) than control mature crowns from plants in the same population.

MATERIALS AND METHODS

Fourteen small regrowth peyote crowns weighing 2–4 grams each (fresh weight) were collected from 14 individual plants in a South Texas population in March 2012. (These samples are hereafter referred to as the "Small Regrowth group".) Biopsy samples of crown tissue (ca. 4 g fresh weight from each individual) were field-collected from 10 mature (eight-ribbed) individual peyote plants in the same population. (These samples are hereafter referred to as the "Mature group".) The cactus tissue from each group was cut into small slices, which were set to dry for a week on a drying rack at room temperature. Once desiccated, the tissue of each group was ground to a fine powder with a mortar and pestle. At that point the individual plant samples in each group were pooled and mixed to constitute a single homogeneous sample to represent each group. A sample of 2.0 g of the ground cactus tissue from each group was then placed in a 200-mL beaker to which 100 mL of HPLC-grade methanol was added. The beaker for each sample was sealed with Parafilm® to prevent evaporation of the methanol, and the beakers were kept at room temperature with daily swirling for a week to effect alkaloid extraction. The methanol extract was evaporated to dryness in the hood at room temperature, and the residue of each extract was dissolved in 200 mL HPLC-grade water. The pH was lowered to approximately 3 with concentrated HCl. The acidified aqueous extract was poured into a 500-ml separatory funnel, to which 50 mL of dichloromethane was added. The separatory funnel was shaken to mix the aqueous and dichloromethane phases thoroughly, and

the aqueous and dichloromethane layers were then allowed to separate overnight. The rationale was that protonated phenethylamines such as mescaline and protonated tetrahydroisoquinolines such as pellotine remain in the acidified aqueous layer, while less polar substances dissolve preferentially in the dichloromethane layer. The latter, containing fats and other nonpolar compounds, was drained out of the separatory funnel and set aside. Two additional defatting extractions of the remaining acidified aqueous phase in the separatory funnel were done, each with 50 mL of dichloromethane, and in each case the aqueous and organic layers were allowed to separate, whereupon the dichloromethane phase was drained from the separatory funnel and discarded.

After the third defatting extraction of the acidified aqueous phase, the aqueous phase was drained from the separatory funnel into a beaker, and its pH was raised from 3 to 12 with sodium hydroxide (5 M), in order to deprotonate close to 100% of the mescaline ($pK_a = 9.5$). The alkalinized aqueous extract was then poured into a separatory funnel, and 50 mL of dichloromethane was added. The rationale was that alkalinizing the aqueous phased in this classic acid-base cleanup procedure was that the deprotonated alkaloids would now be more soluble in the nonpolar dichloromethane than in water. The organic and aqueous phases were mixed well and left to separate. The dichloromethane layer was drained into a 200-mL beaker and saved. Another 50 mL of dichloromethane was then added to the remaining alkaline aqueous phase in the separatory funnel. Upon separation of the organic and aqueous phases, the organic phase was added to the first dichloromethane extract, and the combined extracts were left in a hood at room temperature to evaporate to dryness. The residue, containing the mescaline and related alkaloids, was then redissolved in 10.0 mL of methanol and stored in the freezer in a sealed vial. Samples of the two extracts (Small Regrowth and Mature) were run on an Agilent 1260 Infinity HPLC with a diode-array detector (G4212B) set at 205 nm, using 70% methanol (HPLC grade) as the mobile phase, and a Phenomenex Gemini 5µ C18 column as the stationary phase. Samples of 1.0 µL were injected with a flow rate of 1.0 mL/min and run for 30 minutes. Each of the two samples of alkaloid extract, after appropriate dilution to obtain on-scale HPLC peaks, was run three times, and the values of area under the curve (AUC) of the three runs per sample were averaged. Appropriate dilutions were made of mescaline standard and run on the HPLC three times under the same conditions described above, to create a standard curve. The mean AUC value for each sample of alkaloid extract was then interpolated on the standard curve to yield the corresponding weight (in µg) of mescaline in the HPLC sample of alkaloid extract. From the latter value (one such concentration value per sample group), we calculated the original concentration of mescaline in the homogenized desiccated tissue sample from the Small Regrowth group and in that of the Mature group.

Confirmation of the identity of mescaline in these samples was achieved with GC/MS. A sample methanol extract of *L. williamsii* from this study was evaporated to dryness under an N_2 stream at room temperature, and the residue dissolved in dichloromethane for analysis. The instrumentation was an Agilent 6890 GC equipped with a DB-5ms (0.25 mm I.D. × 30 m) column, splitless injection (250°), and an Agilent 5972 MSD (transfer line at 275°), operated in full scan mode. Helium was used as the carrier, and the oven program was 70° (with a 1-min hold), then 20°/min to 250°, with a final hold. The mescaline peak had an identical retention time to that of an authentic reference sample, and the mass spectrum matched a spectrum in the NIST database (Stein et al. 2005) with 93.5% probability of best match.

RESULTS AND DISCUSSION

Group mescaline concentrations for the Small Regrowth and Mature groups sampled are presented in Table 1. The group values reported are physical averages resulting from the within-group pooling and homogenization of tissue samples of the individual plants sampled and are not statistical means. (A forthcoming manuscript from our lab will report mescaline concentration data on a substantially larger number of individuals in different life stages and size ranges, with statistical analysis of individual values of mescaline concentration in crown tissue.) Mescaline concentrations were 3.80 g/100g tissue in the Mature group and 2.01 g/100 g tissue in the Small Regrowth group, based on tissue samples ≥ 2 g fresh weight from 10 individuals in the Mature group and 14 individuals in the Small Regrowth group. The difference between these levels amounted to a 47% reduction in mescaline concentration in the Small Regrowth group compared to the Mature group. A gas chromatogram

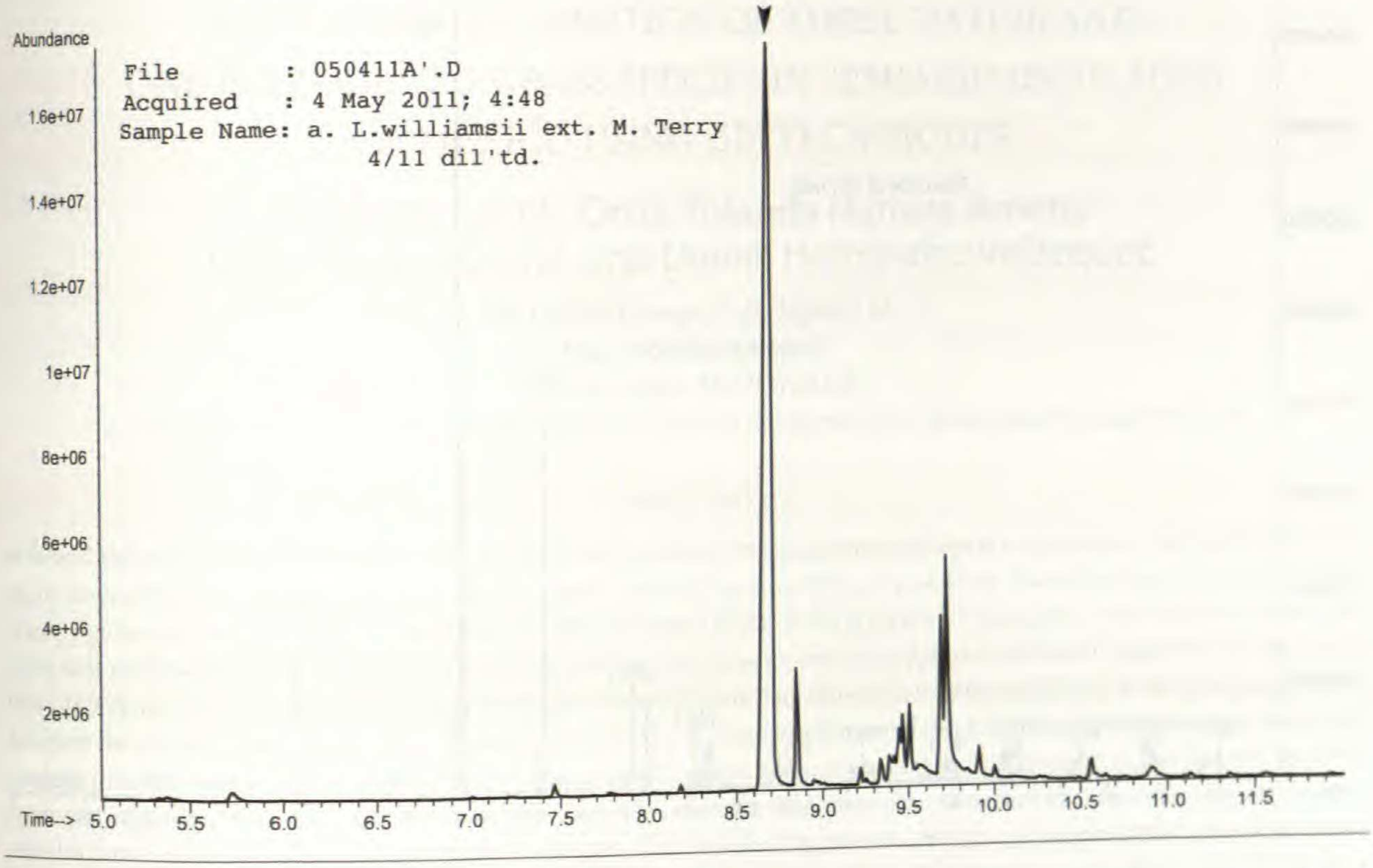

FIG. 2. Gas chromatogram of peyote extract showing prominent mescaline peak.

TABLE 1. Mean mescaline concentration as percentage of mescaline as a constituent of peyote crown tissue (dry-weight basis) in "Mature" peyote crowns and "Small Regrowth" crowns sampled in situ from the same population in South Texas.

Crown Type	Group Mescaline Concentration (as % of dry tissue weight)
Mature	3.80
Small Regrowth	2.01

of peyote extract and a mass spectrum of the most prominent GC peak from peyote extract are presented in Figs. 2 and 3, respectively.

These data support the hypothesis that mescaline concentrations are reduced in small regrowth crowns of peyote in comparison to the concentrations in mature crowns from plants not previously harvested. This confirms the validity of the widespread opinion among NAC members that small regrowth buttons are "weaker" than larger, mature peyote crowns (T. Herrera, pers. obs.). It also adds another dimension to the damage being done by the too frequent harvesting of peyote in South Texas. Mature crowns have been largely replaced by small regrowth crowns in the peyote market. In order to obtain an effective ceremonial dose of the sacrament, an NAC participant in a peyote meeting must consume a substantially greater number of small buttons than would be the case with mature buttons. The finding in the current study that small regrowth buttons are indeed lower in mescaline concentration than mature buttons, favors consumption of still larger numbers of buttons by each participant in an NAC meeting in order to get "enough" peyote to attain the appropriate spiritual state. Such compensatory increased consumption of peyote attributable to reduced mescaline **concentration**—in addition to the increased consumption required to compensate for the small **size** of the regrowth buttons—can only exacerbate the vicious circle of increased harvesting leading to increased consumption leading to increased harvesting. At the population level in South Texas, this vicious circle has been seen (MT & KT, pers. obs.) to progress downward to the bottom of the extinction vortex for *L. williamsii*.

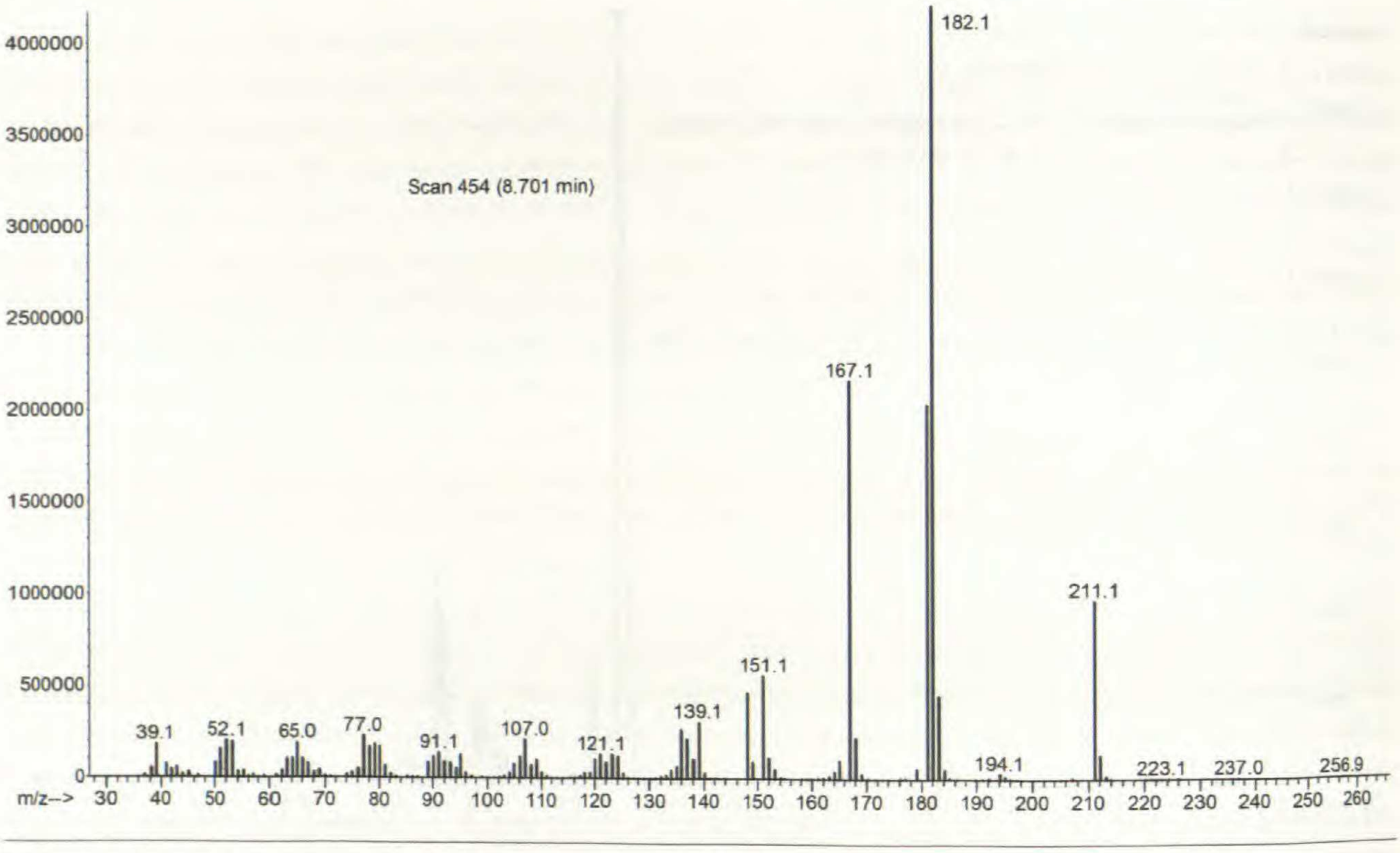

Fig. 3. Mass spectrum of the prominent GC peak in Fig. 2. This spectrum matches that of mescaline with P = 93.5%.

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